

CLAIMS

1. A method of enhancing the growth rate and/or controlling the metabolic activity of a lactic acid bacterial strain, comprising cultivating the strain in association with a lactic acid bacterial helper organism that is defective in its pyruvate metabolism.
2. A method according to claim 1 wherein the cultivation of the lactic acid bacterial strain in association with the helper organism results in an enhancement of the acid production of the strain.
3. A method according to claim 2 wherein the cultivation results in a Δ pH of at least 0.05 after 3 hours or more of cultivation.
4. A method according to claim 1 wherein the strain is cultivated in a medium having an initial degree of oxygen saturation which is 10% or higher.
5. A method according to claim 4 wherein the medium has an initial degree of oxygen saturation which is 20% or higher.
6. A method according to claim 1 wherein the helper organism is capable of reducing the amount of oxygen present in the medium by at least 1% per hour.
7. A method according to claim 1, wherein the helper organism is a derivative of a lactic acid bacterium.
8. A method according to claim 7 wherein the helper organism essentially does not produce lactic acid.
9. A method according to claim 1 wherein the helper organism is defective in its ability to express at least one enzyme

selected from the group consisting of pyruvate formate lyase, pyruvate dehydrogenase, lactate dehydrogenase, acetolactate synthetase, second acetolactate synthetase, acetolactate decarboxylase and diacetyl reductase.

5 10. A method according to claim 9 wherein the helper organism is *Lactococcus lactis* subs. *lactis* strain DN223 deposited under the accession No. DSM 11036.

11. A method according to claim 9 wherein the helper organism is *Lactococcus lactis* subs. *lactis* strain DN224 deposited under
10 the accession No. DSM 11037.

12. A method according to claim 1, wherein the lactic acid bacterial strain is cultivated in a medium which is selected from the group consisting of milk, meat, flour dough, wine and a plant material.

15 13. A method according to claim 1 wherein the ratio between helper organism cells and cells of the lactic acid bacterial strain is in the range of 1000:1 to 1:1000.

14. A method according to claim 1 wherein a gene coding for an enzyme that is capable of regenerating NAD^+ is overexpressed in the helper organism.

15. A method according to claim 14 wherein the enzyme catalyses the reduction of O_2 to H_2O or H_2O_2 .

16. A method according to claim 15 wherein the enzyme is $\text{NADH:H}_2\text{O}$ oxidase including the enzyme having the sequence SEQ
25 ID NO:2.

17. A method according to claim 14 wherein the helper organism is an Ldh^- strain.

18. A method of improving the shelf life and/or the quality of an edible product comprising adding to the product a lactic acid bacterial strain that is defective in its pyruvate metabolism.

5 19. A method according to claim 18 wherein the lactic acid bacterial strain essentially does not produce lactic acid.

20. A method according to claim 18 wherein the lactic acid bacterial strain is defective in its ability to express at least one enzyme selected from the group consisting of pyruvate
10 formate lyase, pyruvate dehydrogenase, lactate dehydrogenase, acetolactate synthetase, second acetolactate synthetase, acetolactate decarboxylase and diacetyl reductase.

21. A method according to claim 18 wherein the edible product is selected from the group consisting of milk, flour dough,
15 meat, wine and a plant material.

22. A method according to claim 21 wherein the edible product is non-pasteurized milk.

23. A method according to claim 18 wherein the lactic acid bacterial strain is added to the product at its site of
20 production.

24. A method according to claim 23 wherein the lactic acid bacterial strain is added to raw milk following milking.

25. A starter culture composition comprising a lactic acid bacterium and a lactic acid bacterial helper organism that is defective in its pyruvate metabolism, said helper organism being capable of enhancing the growth rate and/or controlling the metabolic activity of the lactic acid bacterium.

26. A composition according to claim 25 wherein the helper organism essentially does not produce lactic acid.

27. A composition according to claim 25 wherein the helper organism is defective in its ability to produce at least one enzyme selected from the group consisting of pyruvate formate lyase, pyruvate dehydrogenase, lactate dehydrogenase, acetolactate synthetase, second acetolactate synthetase, acetolactate decarboxylase and diacetyl reductase.

10 28. A composition according to claim 25 wherein the helper organism is *Lactococcus lactis* subs. *lactis* strain DN223 deposited under the accession No. DSM 11036.

29. A composition according to claim 25 wherein the helper organism is *Lactococcus lactis* subs. *lactis* strain DN224 deposited under the accession No. DSM 11037.

30. A composition according to claim 25 wherein a gene coding for an enzyme that is capable of regenerating NAD^+ is overexpressed in the helper organism.

31. A composition according to claim 30 wherein the enzyme catalyses the reduction of O_2 to H_2O or H_2O_2 .

20 32. A composition according to claim 31 wherein the enzyme is $\text{NADH:H}_2\text{O}$ oxidase including the enzyme having the sequence SEQ ID NO:2.

25 33. A composition according to claim 30 wherein the helper organism is an Ldh^- strain.

34. A composition according to claim 25 that comprises two or more different lactic acid bacterial strains.

35. An isolated or non-naturally occurring lactic acid bacterium that is defective in its ability to express lactate dehydrogenase and in which a gene encoding a protein capable of regenerating NAD^+ is overexpressed, wherein the gene capable of regenerating NAD^+ that is overexpressed codes for an enzyme catalysing the reduction of O_2 to H_2O or H_2O_2 .

36. A bacterium according to claim 35 which is defective in its ability to express at least one further enzyme selected from the group consisting of pyruvate formate lyase, pyruvate dehydrogenase, acetolactate synthetase, second acetolactate synthetase, acetolactate decarboxylase and diacetyl reductase.

10 37. A bacterium according to claim 35 wherein the gene capable of regenerating NAD^+ that is overexpressed codes for an enzyme catalysing the reduction of O_2 to H_2O or H_2O_2 .

38. A bacterium according to claim 37 wherein the enzyme is $\text{NADH:H}_2\text{O}$ oxidase including the enzyme having the sequence SEQ ID NO:2.

15 39. An isolated DNA fragment derived from a lactic acid bacterium comprising a gene coding for a polypeptide having $\text{NADH:H}_2\text{O}$ oxidase activity.

40. A DNA fragment according to claim 39 which is selected from the group consisting of the sequence shown in SEQ NO ID:1 and a
20 variant or derivative hereof which is at least 50% identical with said sequence.

41. A recombinant DNA molecule comprising the DNA fragment of claim 39.

42. A recombinant DNA molecule comprising the DNA fragment of claim 40.

43. The bacterium of claim 35 which is selected from the group consisting of bacteria of the genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Propionibacterium*, and *Bifidobacterium*.

44. The bacterium of claim 35 which is a strain of *Lactobacillus lactis*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, or *Lactobacillus delbruecki*, *Leuconostoc mesenteroides*.

45. The bacterium of claim 35 which is a strain of *Lactococcus lactis*.